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(54) Title: STABILISED GROWTH HORMONE FORMULATION AND METHOD OF PREPARATION THEREOF		
(57) Abstract A method for the preparation of a stable, liquid formulation of growth hormone, comprising growth hormone, a buffer and a stabilising effective amount of at least one stabilising agent selected from the group consisting of: (i) polyethylene-polypropylene glycol non-ionic surfactants, (ii) taurocholic acid or salts or derivatives thereof, and (iii) methyl cellulose derivatives, wherein the method comprises admixing the growth hormone with the buffer and the stabilising agent(s) under conditions such that the growth hormone is not exposed to concentrations of the buffer or stabilising agent(s) which are greater than 2x the final concentrations of the buffer or stabilising agent(s) in the formulation.		

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STABILISED GROWTH HORMONE FORMULATION AND METHOD OF PREPARATION THEREOF.

5 FIELD OF THE INVENTION

This invention relates to stabilised growth hormone (GH) formulations and in particular to liquid formulations of human growth hormone (hGH) which are stabilised by the incorporation of stabilising excipients. These liquid formulations of hGH have improved chemical and physical stability. The present invention
10 relates particularly to a method for the preparation of these stabilised GH formulations.

BACKGROUND OF THE INVENTION

The growth hormones of humans and animals are proteins containing
15 approximately 191 amino acids which are found in the anterior pituitary. A major biological action of GH is to promote somatogenesis in young humans and animals and to maintain tissues in older creatures. Organs affected by GH include the skeleton, muscles, connective tissue and the viscera. Growth hormone acts by interacting with specific receptors on the target cell membranes.

20

Human growth hormone (hGH) is a key hormone involved in the regulation of normal human somatic growth and also affects a variety of physiological and metabolic functions, including linear bone growth, lactation and cellular energy use, among others. Deficiency of hGH in young children leads to short stature, and this
25 condition has been treated by exogenous administration of hGH.

In the past, attention has been focused on determining the molecular functions of the growth hormones of various species. Commercial interest has been strong from both medical and veterinary circles, and the hGH gene has been

cloned. Both hGH and a derivative thereof, methionyl-hGH (met-hGH), are now being biosynthetically produced in mammalian and bacterial cell culture systems.

5 In order for hGH to be available commercially as a therapeutic pharmaceutical preparation, stable formulations must be prepared. Such formulations must be capable of maintaining activity for appropriate storage times, they must be readily formulated and be acceptable for administration by patients.

10 Human GH has been formulated in a variety of ways. By way of example, US Patent No 5,096,885 discloses a stable pharmaceutically acceptable formulation of hGH comprising, in addition to the hGH, glycine, mannitol, a buffer and optionally a non-ionic surfactant, the molar ratio of hGH:glycine being 1:50-200. International Patent Publication No. WO 93/19776 discloses injectable formulations of GH
15 comprising citrate as buffer substance and optionally growth factors such as insulin-like growth factors or epidermal growth factor, amino acids such as glycine or alanine, mannitol or other sugar alcohols, glycerol and/or a preservative such as benzyl alcohol. International Patent Publication No. WO 94/01398 discloses a GH formulation containing hGH, a buffer, a non-ionic surfactant and, optionally,
20 mannitol, a neutral salt and/or a preservative.

In European Patent Publication No. 0131864 (and corresponding Australian Patent No. 579016) there is disclosed an aqueous solution of proteins with molecular weight above 8500 daltons, which have been protected from adsorption
25 at interfaces, against denaturing and against precipitation of the protein by addition of a linear polyoxyalkylene chain-containing surface-active substance as a stabilising agent.

European Patent Publication No. 0211601 discloses a growth promoting
30 formulation comprising an aqueous mixture of growth promoting hormone and a

block copolymer containing polyoxyethylene-polyoxypropylene units and having an average molecular weight of about 1,100 to about 40,000 which maintains the fluidity of the growth promoting hormone and its biological activity upon administration. Subsequent European Patent Publication No. 0303746 discloses
5 various other stabilisers for growth promoting hormone in aqueous environments including certain polyols, amino acids, polymers of amino acids having a charged side group at physiological pH and choline salts.

Pharmaceutical preparations of hGH tend to be unstable, particularly in
10 solution. Chemically degraded species such as deamidated or sulfoxylated forms of hGH occur, and dimeric or higher molecular weight aggregated species may result from physical instability (Becker et al (1988) *Biotechnol Applied Biochem* **10**, 326; Pearlman and Nguyen (1989), In D. Marshak and D. Liu (eds), *Therapeutic Peptides and Proteins, Formulations, Delivery and Targetting*, Current
15 Communications in Molecular Biology, Cold-Spring Harbour Laboratory, Cold Spring Harbour, New York, pp 23-30; Becker et al (1987) *Biotechnol Applied Biochem* **9**, 478).

As a consequence of the instability of hGH in solution, pharmaceutical
20 formulations of hGH tend to be presented in lyophilised form, which must then be reconstituted prior to use. Lyophilisation is often used to maintain bioactivity and biochemical integrity of polypeptides under a range of storage conditions where stability in solution is not adequate, however it would be advantageous to avoid lyophilisation as this is a costly and time-consuming production step. Lyophilised
25 formulations of hGH are reconstituted before use, usually by the addition of a pharmaceutically acceptable diluent such as sterile water for injection, sterile physiological saline or an appropriate sterile physiologically acceptable diluent. Reconstituted solutions of hGH are preferably stored at 4°C to minimise chemical and physical degradation reactions, however some degradation will occur during
30 such storage which can be for a period of up to 14 days.

A pharmaceutical formulation of hGH provided in a liquid form, particularly one that maintained stability of hGH over a prolonged period of time, would be particularly advantageous. As described above, current liquid formulations are limited in storage time by the products of chemical and physical degradation reactions that occur during processing and storage. The problems associated with dimer formation have been reported in Becker, *et al.* (1987), *supra.*, and previous attempts to avoid hGH dimer formation have not succeeded.

It is an object of the present invention to provide stable liquid formulations of hGH that do not result in the formation of undesirable aggregated species or cause chemical changes that reduce biological activity or alter receptor recognition. Another object is to provide a formulation that may be delivered via the needleless injector for subcutaneous injection, or aerosolised for pulmonary use.

SUMMARY OF THE INVENTION

According to the present invention, there is provided a method for the preparation of a stable, liquid formulation of growth hormone comprising growth hormone, a buffer and a stabilising effective amount of at least one stabilising agent selected from the group consisting of:

- (i) polyethylene-polypropylene glycol non-ionic surfactants,
- (ii) taurocholic acid or salts or derivatives thereof, and
- (iii) methylcellulose derivatives,

wherein the method comprises admixing the growth hormone with the buffer and the stabilising agent(s) under conditions such that the growth hormone is not exposed to concentrations of the buffer or stabilising agent(s) which are greater than 2x the final concentrations of the buffer or stabilising agent(s) in the formulation.

The present invention also extends to a stable, liquid formulation of growth hormone, prepared by the method as broadly described above.

In yet another aspect, the invention also extends to a stable, liquid
5 formulation of growth hormone, comprising growth hormone, a buffer and a stabilising effective amount of at least one stabilising agent selected from taurocholic acid or salts or derivatives thereof, and methyl cellulose derivatives.

Throughout this specification, unless the context requires otherwise, the word
10 "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

In a particularly preferred embodiment, this invention provides a method for
15 the preparation of a stabilised, pharmaceutically acceptable liquid formulation of human growth hormone comprising:

- (a) a pharmaceutically effective amount of hGH,
- (b) 0.01-5.0% w/v of at least one stabilising agent selected from the group broadly defined above, and
- 20 (c) a pharmacologically acceptable buffer.

Preferably, this formulation comprises 0.05-2.0% w/v, more preferably 0.08-1.0% w/v, of the stabilising agent(s).

25 Particularly preferred stabilising agents are Pluronic polyols, taurocholic acid and its salts, and hydroxypropylmethyl cellulose.

The stabilised, liquid formulation of growth hormone preferably also contains a pharmacologically acceptable buffer such as a phosphate or citrate buffer, at a
30 concentration of 2.5-50mM, most preferably 10-20mM.

The pH of the formulation is preferably from 5.0 to 7.5, more preferably from 5.0 to 6.8, even more preferably from 5.2 to 6.5, and most preferably from 5.4 to 5.8.

5 In the preparation of the stabilised, liquid formulation of growth hormone, the growth hormone is admixed with the buffer under conditions such that the growth hormone is not exposed to buffer concentrations greater than 2x the final concentration of buffer in the formulation, and subsequently the stabilising agent(s) is added to the admixture under corresponding conditions.

10

In a particularly preferred method of preparation of the stabilised, liquid formulation of growth hormone, exposure of growth hormone is restricted to concentrations of phosphate or citrate buffer and Pluronic polyols not greater than 2x the final concentration of each component.

15

The present invention also extends to a method for treatment of a human or animal patient in need of growth hormone, which comprises administration to said patient of a pharmaceutically effective amount of a stable, liquid formulation of growth hormone as broadly described above.

20

The GH liquid formulation may be administered by bolus injection, with an aerosol device or needleless injector gun or by continuous IV infusion.

25 In the present context, references to "growth hormone" are intended to include all species of GH including human, bovine, porcine, ovine and salmon, among others, particularly hGH, as well as biologically active derivatives of GH. Derivatives of GH are intended to include GH of human or animal species with variations in amino acid sequence, such as small deletions of amino acids or replacement of amino acids by other amino acid residues. Also included are
30 truncated forms of GH and derivatives thereof, as well as GH with amino acid

additions to the amino- or carboxyl-terminal end of the protein, such as methionyl-hGH. Another type of hGH modification is that formed through the covalent addition of polyethylene glycol to reactive hGH amino acids (Davis *et al.*, US Patent 4,179,337).

5

DETAILED DESCRIPTION OF THE INVENTION

The method of preparation of liquid formulations of GH and stabilising agents provided by the present invention results in a stable liquid GH formulation suitable for prolonged storage at temperatures below freezing and above freezing, and for
10 therapeutic administration. Therapeutic formulations containing these stabilising agents are stable, while still allowing therapeutic administration of the formulation.

According to a preferred embodiment of the present invention the GH is hGH.

15

(1) Human Growth Hormone Compositions.

The terms "human growth hormone" or "hGH" denote human growth hormone produced, for example, by extraction and purification of hGH from natural sources, or by recombinant cell culture systems. The sequence of hGH and its
20 characteristics are described, for example, in Hormone Drugs, Gueriguigan et al, USP Convention, Rockville, MD (1982). As described above, the terms also cover biologically active human growth hormone equivalents that differ in one or more amino acids in the overall sequence of hGH, including in particular met-hGH. The terms are also intended to cover substitution, deletion and insertion amino acid
25 variants of hGH or post translational modifications. The hGH used in the formulations of the present invention is generally produced by recombinant means as previously discussed.

A "pharmaceutically effective amount" of GH, particularly hGH, refers to that
30 amount which provides therapeutic effect in various administration regimens. The

compositions of the present invention may be prepared containing amounts of GH at least about 0.1 mg/ml up to about 20 mg/ml or more, preferably from about 1 mg/ml to about 10 mg/ml, more particularly from about 1 mg/ml to about 5 mg/ml.

5

(2) Buffer and pH

The buffer may be any pharmaceutically acceptable buffering agent such as phosphate, tris-HCl, citrate and the like. The preferred buffer is a phosphate or citrate buffer. A buffer concentration greater than or equal to 2 mM and less than 10 50mM is preferred, most advantageously 10-20mM. Suitable pH ranges, adjusted with buffer, for the preparation of the formulations hereof are from about 5 to about 7.5, most advantageously about 5.6. The formulation pH should be less than 7.5 to reduce deamidation of GH.

15 (3) Stabilising Agents

In accordance with the present invention, the formulation contains one or more stabilising agents for enhanced GH stability. The stabilising agent may be a polyethylene-polypropylene glycol nonionic surfactant such as a Pluronic polyol, for example, Pluronics F127, F68, L64, PE6800 and PE6400, a bile salt such as a 20 taurocholic acid salt or derivative thereof, or a methylcellulose derivative such as hydroxypropylmethylcellulose (HPMC). The formulation may contain a single stabilising agent or a combination of two or more thereof.

The concentration of stabilising agent(s) added will be determined by the 25 selection of buffer and pH, but advantageously would be in the range of 0.01% to 5.0%, more preferably 0.05 to 2.0% and even more preferably 0.08 to 1.0%, on a weight to volume basis. The use of stabilising agents improves formulation stability when subjected to prolonged storage over a range of temperatures, including below freezing and above freezing, or when the formulation is subjected to interfacial 30 stress.

The stabilising agent(s) improve formulation stability to interfacial stress with increasing concentration. However, increased stabilising agent(s) concentration reduces chemical stability. In accordance with the present invention, the concentration of stabilising agent(s) is optimised to achieve high stability to
5 interfacial stress with minimum additional chemical instability.

(4) Preferred Formulation of Stabilising Agents and hGH

In the preparation of a formulation in accordance with the present invention, one or more stabilising agents are added to a hGH liquid formulation. As described
10 above, during formulation, the growth hormone is exposed to buffer concentrations no greater than 2x the final concentration of buffer, and preferably the stabilising agent(s) are added to the formulation immediately prior to final volume adjustment. The resulting formulations have enhanced stability to denaturation and are not susceptible to undesirable reactions that may be met during processing and
15 storage. As used herein, the term processing includes filtration, filling of hGH solutions into vials and other manipulations involved in production of the formulations.

Liquid formulations of hGH for therapeutic administration may be prepared
20 by combining hGH and stabilising agents having the desired degree of purity with physiologically acceptable excipients, buffers or preservatives (*Remington's Pharmaceutical Sciences*, 16th Edition, Osol, A. Ed (1980). Acceptable excipients are those which are nontoxic to the patient at the concentrations and dosages employed, and include buffers, preservatives, antioxidants, pH and tonicity
25 modifiers.

The liquid formulation of growth hormone may also include one or more other stabilising excipients if desired. Additional stabilising excipients may include, for example, amino acids such as glycine or alanine, mannitol or other sugar alcohols,

or glycerol. In addition, the liquid formulation may include other growth factors such as insulin-like growth factors or epidermal growth factor.

The preferred embodiment of the invention provides a means for effectively
5 stabilising hGH. The preferred formulation contains one or more stabilising agents selected from Pluronic polyols, taurocholic acid or salts or derivatives thereof, and methylcellulose derivatives. The formulation preferably contains substantially pure hGH free of contaminating peptides or proteins or infectious agents found in humans. Formulations of this preferred embodiment may additionally contain
10 pharmaceutically acceptable additives. These include, for example, buffers, isotonicity and pH modifiers, chelating agents, preservatives, antioxidants, cosolvents and the like, specific examples of these could include citrate salts, phosphate salts and the like. A preservative may be added where the anticipated use of the formulation may compromise sterility, and in such a case a
15 pharmaceutically acceptable preservative such as benzyl alcohol or phenol may be used.

The increased stability of hGH provided by the formulation prepared in accordance with the present invention permits a wider use of hGH formulations that
20 may be more concentrated than those commonly in use in the absence of stabilising agents. For example, stabilised hGH liquid formulations also reduce the incidence of surface induced denaturation of hGH that occurs during aerosolisation or needleless injection of an hGH formulation. Further optimal dispensing of the hGH formulations may be made wherein the hGH formulations of the present invention
25 are dispensed into vials at 1-50mg/vial, preferably 2-25 mg/vial, and more preferably 3-10mg/vial. The increased stability of hGH formulations permits long term storage at an appropriate temperature, such as below freezing (most preferably at -20°C), or above freezing, preferably at 2-8°C, most preferably at 4°C.

Formulations of hGH to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

Therapeutic hGH liquid formulations generally are placed into a container
5 having a sterile access port, for example, an intravenous solution bag or vial having a stopper which can be pierced by a hypodermic injection needle.

The route of administration of the hGH liquid formulations in accordance with the present invention is in accord with known practice, e.g. injection or infusion by
10 intravenous, intraperitoneal, intracerebral, intramuscular, intraocular, intraarterial, or intralesional routes, or by continuous IV infusion.

Further features of the present invention will be apparent from the following Examples, and the accompanying drawings.

15

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the chemical stability of hGH (1.5 mg/ml) in 5 mM phosphate buffer, pH 6.0-7.5.

20

Figure 2 shows the dependence of aggregation of hGH (2 mg/ml in 10mM acetate buffer, pH 4.1-4.5 or 5 mM phosphate buffer, pH 6.0-7.5) induced through interfacial stress (vortex agitation) on solution pH.

Figure 3 summarises graphically the ability of various stabilising agents to
25 reduce the precipitation of aggregated hGH induced through interfacial stress (vortex agitation).

Figure 4 shows stability of two hGH (5 mg/ml) formulations which differ only in the method of introducing the hGH to the excipients, and a third hGH
30 formulation to which 0.005% w/v EDTA has been added.

EXAMPLES

EXAMPLE 1 Methods for Screening Stabilising Agents

5 The ability of stabilising agents to reduce or prevent GH and in particular hGH aggregation in response to interfacial stress has been evaluated using a rapid aggregation method and analysed by size exclusion chromatography (SEC). Chromatography of hGH was conducted using two TSK G3000SW columns (7.8 mm i.d. x 300 mm, Toyo Sodo, Japan) in series. The mobile phase consisted of 0.1 M phosphate, pH 7.0 buffer and was pumped at a flow rate of 0.9 ml/min. Elution of hGH was detected by UV absorbance at 214 nm using a sample volume of 20 μ l.

15 The rapid aggregation method involved the introduction of a high air/water interface by vortex agitation of hGH solutions at constant speed for 15-60s in capped polypropylene tubes (11 mm i.d. x 74 mm). Samples were equilibrated for 30 min at room temperature to allow precipitation to proceed, then were filtered through 0.2 μ m cellulose acetate microcentrifuge filters and the filtrate was analysed by SEC. Control solutions of each sample that did not receive treatment were included in SEC analysis.

25 The amount of total soluble hGH remaining (peak area of monomeric and higher molecular weight species) was expressed as a percentage of the total peak area (due to hGH) of the appropriate untreated control solution.

Table 1 shows the effect of various stabilising agents on the extent of aggregation of hGH induced by interfacial stress at pH 7.0.

30 Table 2 shows the effect of various stabilising agents on the extent of aggregation of hGH induced by interfacial stress at pH 6.0.

Table 3 shows the effect of various stabilising agents on the extent of aggregation of hGH induced by interfacial stress at pH 5.6.

Table 4 shows the effect of isotonicity adjustment on the extent of
5 aggregation of hGH (1.5 mg/ml) in various buffers at pH 5.6.

Table 5 shows the effect of various stabilising agents on the extent of aggregation of hGH induced by freeze-thawing at pH 5.6.

10 As shown in the accompanying tables, a number of excipients were very effective at reducing or preventing aggregation of hGH induced by interfacial stress. Pluronic polyols provided near quantitative protection at concentrations above 0.05% w/v with monomeric hGH only remaining. Taurocholate, provided near
15 quantitative protection at concentrations above 0.02% w/v with monomeric hGH only remaining. Taurodeoxycholate was not suitable as a stabiliser at a pH of 5.6 as it caused dimerisation of hGH in the absence of interfacial stress.

TABLE 1 Aggregation of hGH (1.5 mg/ml) in the presence of excipients at pH 7.0 induced by interfacial stress.

Excipient	Concentration (mM) (% w/v)		% Total Soluble hGH Remaining (n=2, SEC analysis)	Species of hGH Remaining
buffer	control		7.0 ± 1.15	(mean ± sd, n=5)
Pluronic Polyols:				
Pluronic F-127	1.4	1.75	100, 99.8	monomer only in all samples
	3.2	4.0	100, 100	
Taurocholic Acid Derivatives:				
Taurocholate	1.3	0.07	75.3, 68.5	monomer only in all samples
	5	0.27	100, 100	
	75	4.0	100, 100	
Taurodeoxycholate	0.4	0.02	13.7, 15.0	monomer only in all samples
	2	0.11	100, 100	
	30	1.6	100, 100	
Methyl cellulose derivatives:				
Hydroxypropylmeth ylcellulose (HPMC)	0.01	0.1	44.1, 47.1	monomer only in all samples
	0.05	0.5	98.9, 99.4	

TABLE 2 Aggregation of hGH (1.5 mg/ml) in the presence of excipients at pH 6.0 induced by interfacial stress.

Excipient	Concentration (mM) (% w/v)		% Total Soluble hGH Remaining (n=2, SEC analysis)	Species of hGH Remaining
buffer	control		2.4 ± 2.05	(mean ± sd, n=6)
Pluronic Polyols:				
Pluronic F-127	0.01	0.01	3.34, 1.12	monomer only in all samples
	0.04	0.05	93.7, 91.9	
	0.08	0.1	100, 100	
	0.4	0.5	100, 99.6	
	1.6	2.0	98.5, 97.7	
Pluronic F-68	0.01	0.01	1.2, 2.1	monomer only in all samples
	0.06	0.05	86.4, 97.5	
	0.12	0.1	98.6, 100	
	0.6	0.5	100, 99.7	
Pluronic L-64	0.03	0.01	1.1, 1.4	monomer only in all samples
	0.17	0.05	93.7, 79.4	
	0.35	0.1	100, 100	
	1.7	0.5	100, 98.4	
Pluronic PE-6800	0.12	0.1	98.7, 99.2	monomer only in all samples
	0.6	0.5	99.6, 99.6	
	2.4	2.0	100, 99.0	
Pluronic PE-6400	0.35	0.1	100, 100	monomer only in all samples
	1.7	0.5	100, 100	
Taurocholic Acid Derivatives:				
Taurocholate	1.3	0.07	16.8, 11.9	monomer only in all samples
	6	0.34	98.5, 100	
	15	0.84	97.8, 99.3	
	25	1.4	98.7, 99.1	
Taurodeoxycholate	0.4	0.02	9.2, 4.9	approximately 0.5% dimer
	2	0.10	22.6, 22.0	
	6	0.31	87.2, 88.3	
	12	0.63	99.5, 100	
Methyl Cellulose Derivatives:				
HPMC	0.01	0.1	28.6, 24.0	monomer only in all samples
	0.03	0.25	58.6, 63.4	
	0.04	0.4	91.9, 93.6	

TABLE 3 Aggregation of hHG (1.5 mg/ml) in the presence of excipients at pH 5.6 induced by interfacial stress

Excipient	Concentration (mM) (% w/v)		% Total Soluble hGH Remaining (n=2, SEC analysis)	Species of hGH Remaining
buffer	control		0.79, 1.16	(mean ± sd, n=2)
Pluronic Polyols:	0.04	0.05	69.6, 53.8	2% dimer present 0.7% dimer present monomer only
Pluronic F-127	0.08	0.1	99.5, 99.7	
	0.4	0.5	99.5, 98.8	
Pluronic F-68	0.06	0.05	69.9, 66.8	monomer only in all samples
	0.12	0.01	99.3, 98.1	
	0.6	0.5	99.8, 100	
Taurocholic Acid Derivatives:				
Taurocholate	5	0.27	99.7, 99.2	monomer only in all samples
	10	0.54	100, 100	
	25	1.4	99.4, 100	
Taurodeoxycholate	2	0.10	95.7, 91.8*	7% dimer present 27% dimer present 34% dimer present
	6	0.32	92.1, 87.8*	
	12	0.63	91.6, 84.4*	
Methyl Cellulose Derivatives:				
HPMC	0.01	0.1	5.8, 2.1	monomer only in all samples
	0.03	0.25	34.8, 36.7	
	0.04	0.4	88.8, 88.4	

* in the presence of taurodeoxycholate, dimerisation occurred in the absence of interfacial stress

Aggregation characteristics of hGH (1.5 mg/ml, pH 5.6) in citrate or phosphate (5 or 20 mM) buffers with or without added sodium chloride (to isotonicity) were investigated as aggregation has been reported to be dependent

on phosphate concentration (Pearlman and Nguyen, 1992, *J.Pharm. Pharmacol.* 44: 178-185).

The experimental method as described previously was followed with modification of treatment time (15 sec).

TABLE 4 Aggregation of hGH (1-5 mg/ml) in various buffer systems at pH 5.6.

Buffer system	% Total Soluble hGH Remaining ^a		Species of hGH present
	no added NaCl	added NaCl	
5 mM phosphate	— ^b	44.5, 30.3	monomer only
5 mM citrate	18.9, 21.2	44.3, 46.4	monomer only
20 mM phosphate	28.5, 33.1	43.1, 38.7	monomer only
20 mM citrate	24.6, 33.2	55.5, 50.0	monomer only

a monomer plus higher molecular weight species

b insufficient hGH solubility.

Aggregation of hGH was not found to be dependent on the nature of the buffer or buffer concentration. Aggregation of hGH was inversely related to ionic strength (when adjusted with NaCl).

Aggregation of hGH (1.5 mg/ml in 20 mM isotonic citrate buffer, pH 5.6) in the presence of excipients induced by freeze-thawing was investigated. Samples of hGH (100 µl) in the presence of various excipients were frozen at -20°C for 24 hr then thawed at room temperature and equilibrated for 30 min to allow precipitation to proceed. Analysis of filtered samples was conducted by SEC as described previously.

TABLE 5 Aggregation of hGH (1.5 mg/ml) in the presence of excipients at pH 5.6 induced by freeze-thawing.

Excipient	Concentration (mM) (% w/v)		% Total Soluble hGH Remaining (n=2, SEC analysis)	Species of hGH Remaining
buffer	control		98.8, 95.5	monomer only
Pluronic Polyols:				
Pluronic F-127	0.08	0.1	95.9, 97.5	monomer only
Pluronic F-68	0.12	0.1	100, 100	monomer only
Taurocholic Acid Derivatives:				
Taurocholate	5	0.27	97.9, 100	monomer only
Taurodeoxycholate	2	0.1	95.7, 91.8	contains 24% dimer
MethylCellulose Derivatives:				
HPMC	0.03	0.25	97.2, 97.3	monomer only

Aggregation of hGH after freeze-thawing was not extensive but was not increased by the addition of excipients.

EXAMPLE 2

5 **Figure 1** is a representative profile of the chemical stability of hGH (1.5 mg/ml) in 5 mM phosphate buffer, pH 6.0 - 7.5 (stored at 40°C). Degraded samples were analysed by reversed-phase high performance liquid chromatography (RP-HPLC) according to the method described in the United States Pharmacopoeia (USP 1990) using a Vydac C-4 column. Degradation

10 products were identified according to the method described in U.S. Pharmacopeial Previews, Nov-Dec, 1990, as desamido-hGH or oxidised hGH. The amount of native hGH (Panel A), desamido-hGH (Panel B) and oxidised-hGH (Panel C) present in a degraded sample was expressed as a percentage of peak area (for

native hGH or degraded species) relative to the total peak area (due to hGH) for pH 6.0 (○), pH 6.5 (●), pH 7.0 (▽) and pH 7.5 (▼).

Loss of native hGH was found to follow first order kinetics in the pH range 5 6.0 - 7.5 and Arrhenius behaviour in the temperature range of 8-40°C. The first order rate constants at 40°C were found to range from $2.4 \times 10^{-2} \text{ day}^{-1}$ at pH 6.0 to $7.4 \times 10^{-2} \text{ day}^{-1}$ at pH 7.5. Deamidation and oxidation were the major routes of degradation of hGH consistent with published reports (Pearlman and Nguyen, 1989, supra). Desamido-hGH formed at a faster rate than oxidised hGH. Chemical 10 stability was enhanced at a pH value of 6.0 or below.

Figure 2 shows aggregation and precipitation of hGH (2 mg/ml) in 10 mM acetate buffer (pH 4.1-4.5) or 5 mM phosphate buffer (pH 6.6-7.5) induced through interfacial stress using methods as described in Example 1. The amount of 15 monomeric hGH (peak area due to monomer) or total soluble hGH (peak area due to monomer plus higher order aggregated species) remaining was expressed as a percentage relative to the peak area of the appropriate untreated control solution. The data represent the amount of soluble monomeric hGH (Panel A) or total soluble hGH (Panel B) remaining after vortexing for 30 s (○) or 60 s (●).

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Aggregation and subsequent precipitation of hGH was maximal in the region of pH 5 to 6. Only monomeric hGH remained in solution after interfacial stress in the pH range of 4.1-6.0. Soluble aggregated species (dimer and higher order aggregates) were present mainly in the pH range of 7.0-7.5.

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Figure 3 shows the effect of excipients (% w/v) on aggregation of hGH (1.5 mg/ml in 5 mM phosphate buffer, pH 5.6) induced through interfacial stress by vortexing at constant speed for 60 s as described in Figure 2. The data represent the percentage of total soluble hGH (monomer plus higher order aggregated 30 species) remaining after treatment expressed as a percentage relative to the peak

area due to hGH from SEC analysis in the appropriate control solution in the presence of Pluronic F-68 (○), Pluronic F-127 (●), sodium taurocholate (▽) or HPMC (▼).

- 5 In the absence of excipients, less than 1% hGH remained in solution. Addition of Pluronic polyols, taurocholate or HPMC resulted in a substantial increase in soluble hGH remaining. Pluronic F-68 and F-127 and taurocholate, in particular, provided near quantitative protection of hGH against aggregation.

10 **EXAMPLE 3**

- Figure 4** shows effect of method of formulation on stability of hGH formulations. Formulation 1 was prepared by concentrating purified hGH solution to 7-7.5 mg/mL and adding a two-fold concentrate of a solution containing all the excipients adjusted to a pH which produces a liquid formulation of pH 5.6 without
15 further adjustment, and a final adjustment with water to achieve a final hGH concentration of 5 mg/ml.

- Formulation 2 was prepared by buffer exchange, and purified hGH solution was concentrated to the desired concentration by exchange into a buffer which
20 contained all the excipients (except Pluronic F-68) at the required concentration. Sufficient solid Pluronic F-68 was then added to give the required concentration. The pH was then checked and adjusted if necessary.

 Formulations 1 and 2 have the same specifications:

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hGH (Somatropin)	5 mg
citric acid monohydrate	2.04 mg/mL
trisodium citrate dihydrate	2.85 mg/mL
sodium chloride	6.23 mg/mL
30 sodium hydroxide	0.388 mg/mL

- 21 -

benzyl alcohol	0.9%
Pluronic F-68	0.08%
pH	5.6

- 5 Formulation 3 was prepared as for formulation 2 to the same specifications as formulations 1 and 2, with the addition of 0.005% w/v EDTA.

10 Formulations 1 to 3 were stored at 40°C and tested for hGH content by size exclusion HPLC at intervals over 40 days. As shown in Figure 4, formulation 2 and 3 showed superior stability, particularly in comparison with formulation 1, in this accelerated stability test at 40°C.

CLAIMS:

1. A method for the preparation of a stable, liquid formulation of growth hormone, comprising growth hormone, a buffer and a stabilising effective amount of at least one stabilising agent selected from the group consisting of:
 - (i) polyethylene-polypropylene glycol non-ionic surfactants,
 - (ii) taurocholic acid or salts or derivatives thereof, and
 - (iii) methyl cellulose derivatives,wherein the method comprises admixing the growth hormone with the buffer and the stabilising agent(s) under conditions such that the growth hormone is not exposed to concentrations of the buffer or stabilising agent(s) which are greater than 2x the final concentrations of the buffer or stabilising agent(s) in the formulation.
2. A method according to claim 1, wherein the growth hormone is human growth hormone.
3. A method according to claim 1 or 2, wherein the formulation comprises 0.1-5.0% w/v, preferably 0.05-2.0% w/v, more preferably 0.08-1.0% w/v, of the stabilising agent(s).
4. A method according to any of claims 1 to 3, wherein the stabilising agent(s) are selected from Pluronic polyols, taurocholic acid and its salts, and hydroxypropyl-methyl cellulose.
5. A method according to any of claims 1 to 4, wherein the formulation comprises 0.08% w/v of a Pluronic polyol as the stabilising agent(s).

6. A method according to any of claims 1 to 5, wherein the formulation comprises a pharmacologically acceptable buffer, preferably a phosphate or a citrate buffer, at a concentration of 2.5 to 50 mM, preferably 10-20 mM.
7. A method according to any of claims 1 to 6, wherein the pH of the formulation is from 5.0 to 7.5, preferably 5.0 to 6.8, more preferably from 5.2 to 6.5, and most preferably from 5.4 to 5.8.
8. A method according to any of claims 1 to 7, wherein the stabilising agent(s) is added to the formulation immediately prior to final volume adjustment.
9. A stable, liquid formulation of growth hormone, prepared by a method according to any of claims 1 to 8.
10. A stable, liquid formulation of growth hormone, comprising growth hormone, a buffer and a stabilising effective amount of at least one stabilising agent selected from taurocholic acid or salts or derivatives thereof, and methyl cellulose derivatives.
11. A method of treatment of a human or animal patient in need of growth hormone, which comprises administration to said patient of a pharmaceutically effective amount of a formulation according to claim 9 or claim 10.

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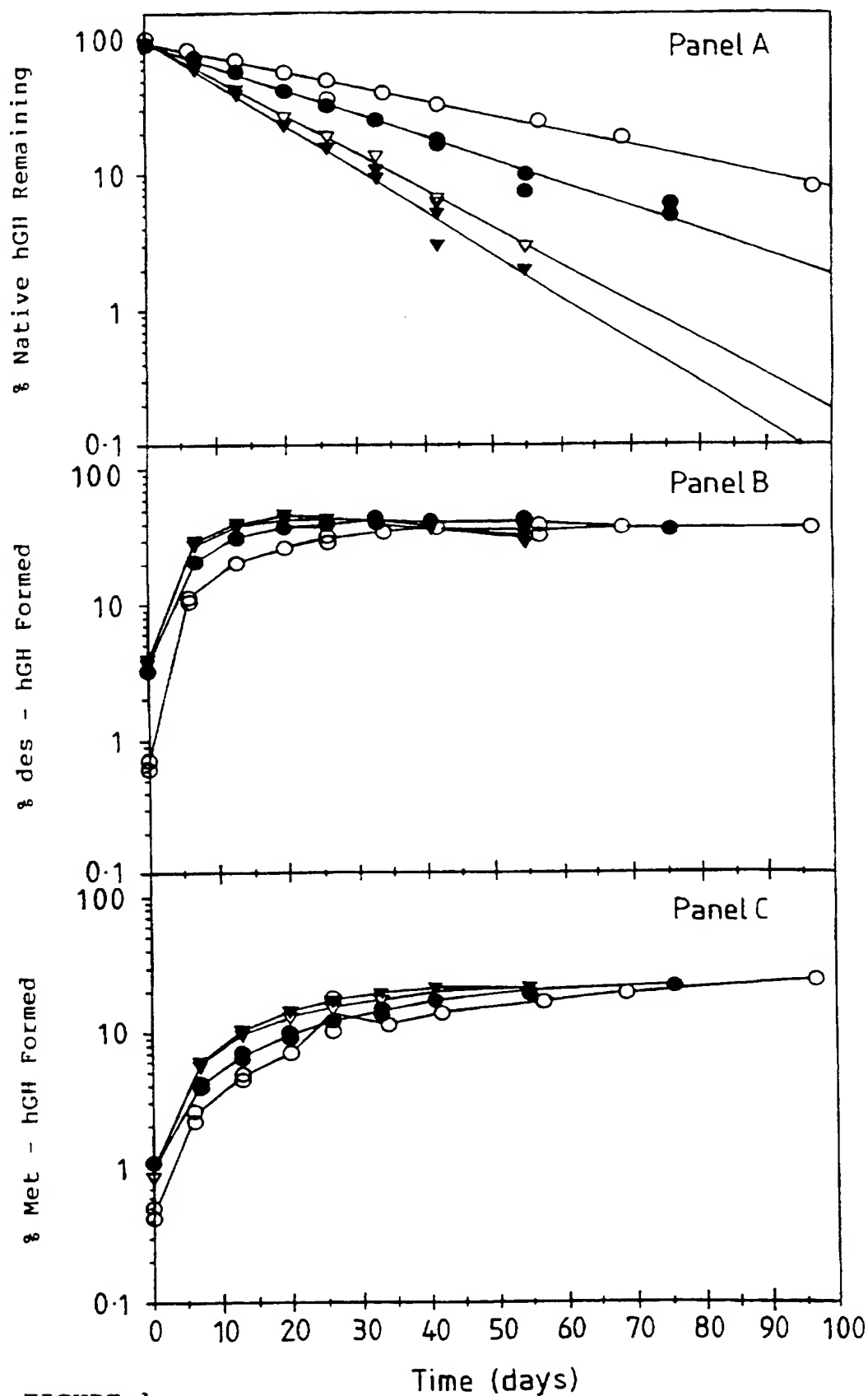
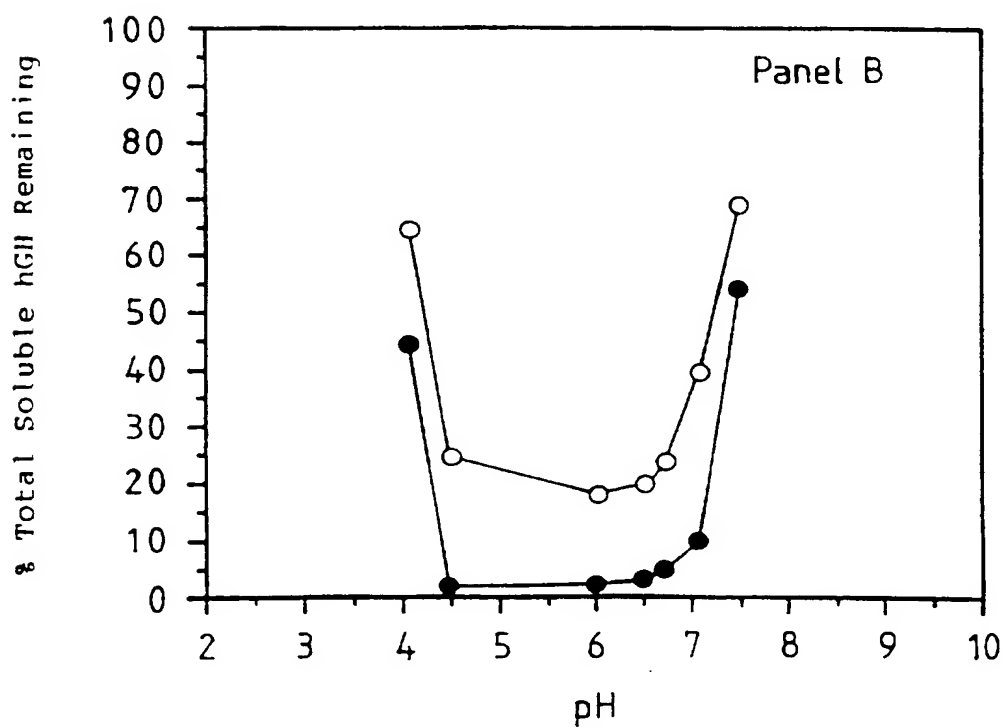
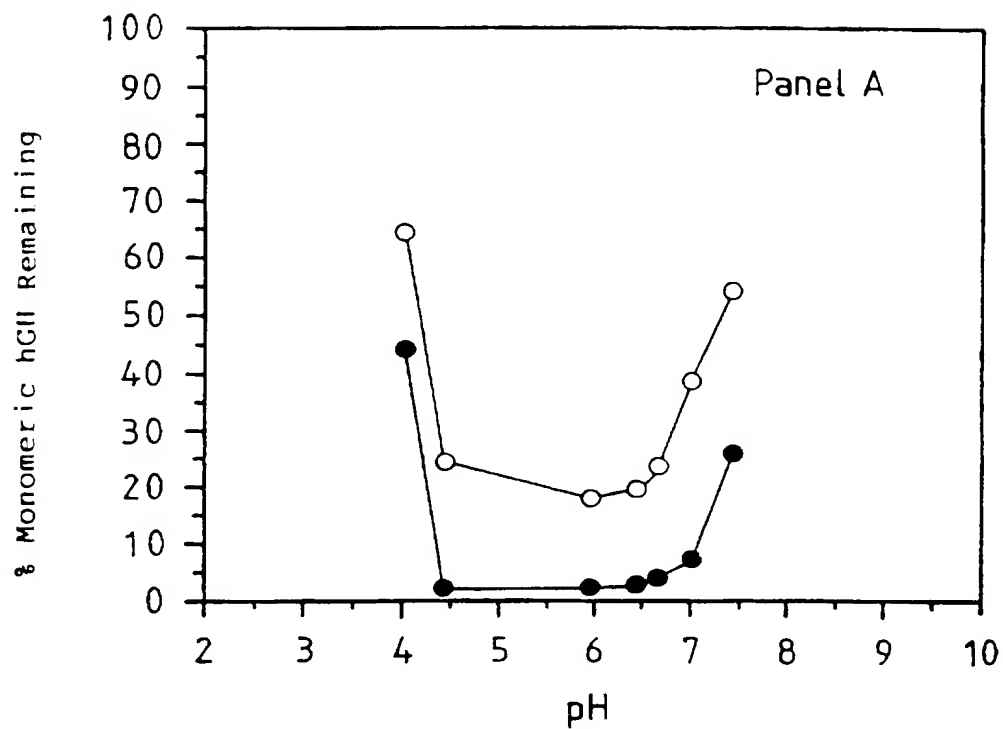


FIGURE 1

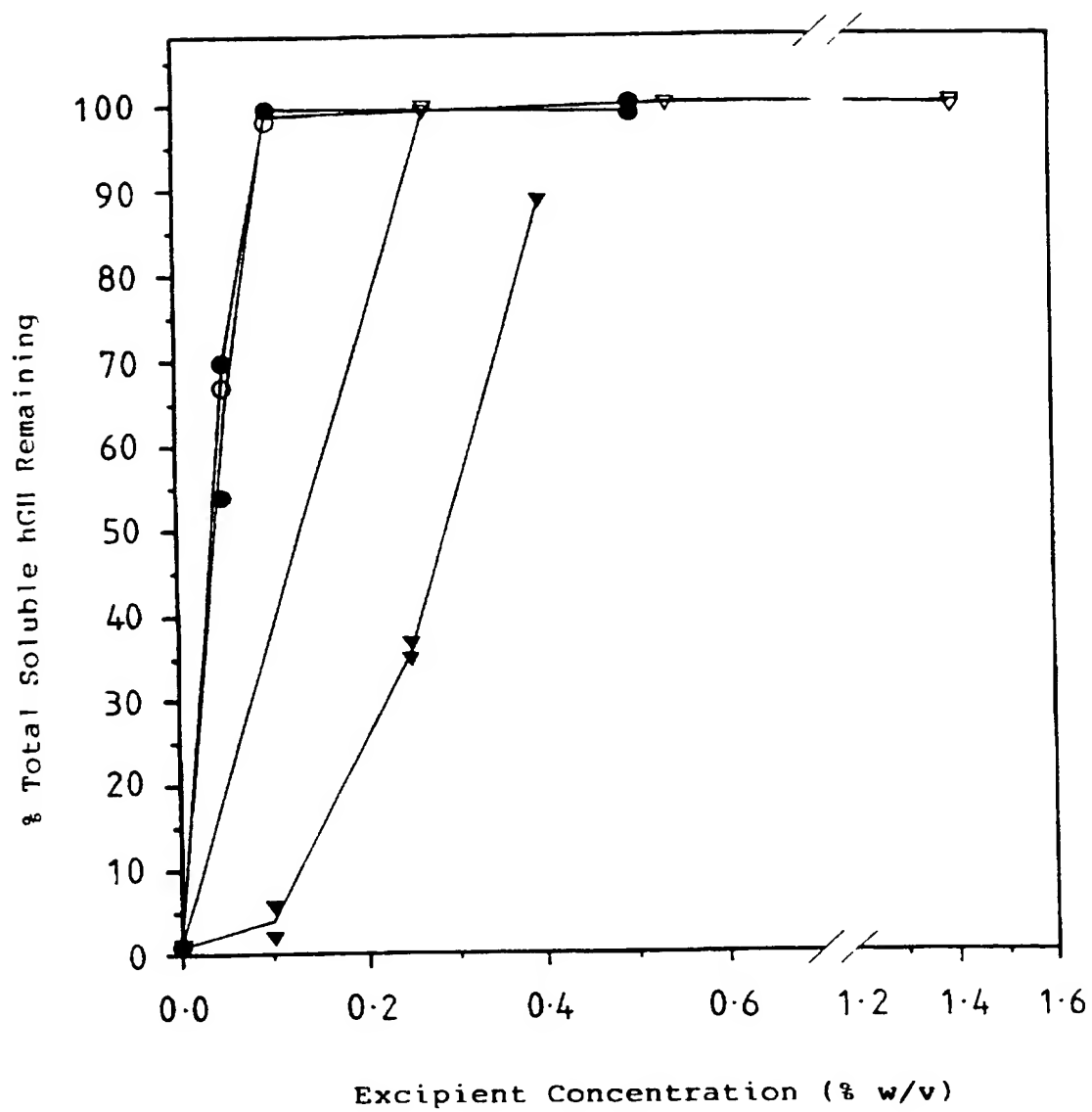
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FIGURE 2



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FIGURE 3

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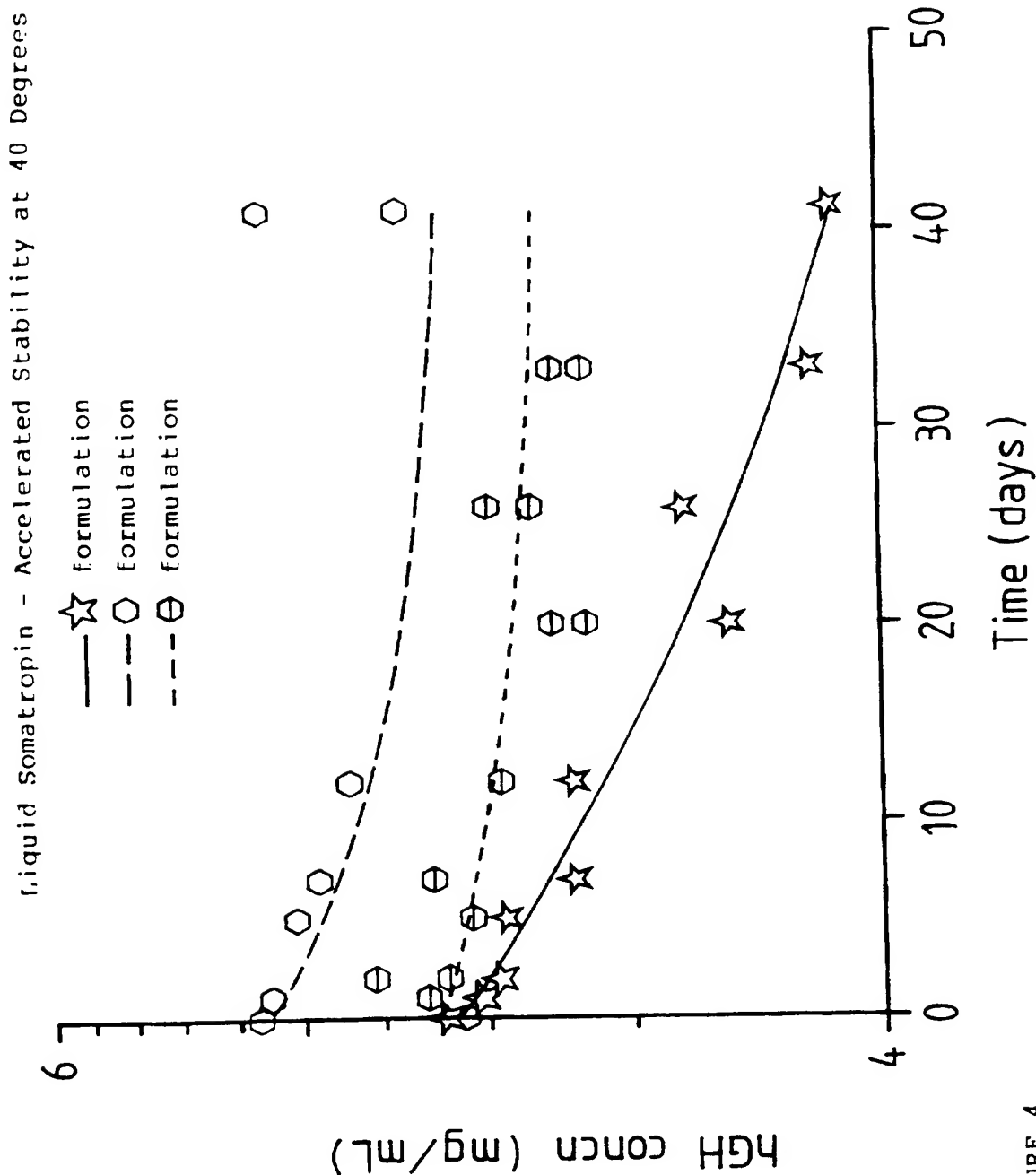


FIGURE 4

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/AU 97/00075

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl^o: A61K 38/27

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC: A61K 38/27

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

AU:IPC as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPAT: A61K 38/27, A61K 37/36, Growth Hormone or GH, Pluronic or Polyethylene () Polyethylene () Glycol, Taurocholic: or Choleic or Cholytaruine or Taurodeoxycholate, Methylcellulose or Hydroxymethylcellulose or HPMC CASM: Same keywords as above

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
<u>X</u> A	AU 60733/86 A (International Minerals and Chemical Corp.) 5 February 1987 Pages 4-5, claims.	<u>1-9</u> 10-11
<u>X</u> Y	Patent Abstracts of Japan, C-846, page 85, JP, A, 3-86834 (Eisai Co Ltd) 11 April 1991	<u>10-11</u> 1-9
<u>X</u> Y	AU 42432/89 A (Patralan Limited) 5 April 1990. Claims 39-44	<u>10-11</u> 1-9

☒ Further documents are listed in the continuation of Box C

☒ See patent family annex

<p>* Special categories of cited documents:</p>	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier document but published on or after the international filing date	"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search
25 March 1997

Date of mailing of the international search report

16 APR 1997

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INTERNATIONAL SEARCH REPORT

International Application No.

PCT/AU 97/00075

C (Continuation)

DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
<u>X</u> Y	US 5283236 A (Orbon Corporation) 1 February 1994. Column 9, lines 1-16, Claims.	<u>10-11</u> 1-9
<u>X</u> Y	AU 20051/88 A (Pierrel SPA) 2 February 1989 Claims.	<u>10-11</u> 1-9
A	Katakam M. etal. Journal of Pharmaceutical Sciences (1995) 84, 713-716.	1-11

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No.

PCT/AU 97/00075

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report			Patent Family Member			
AU	8660733	EP 211601	JP 62070319	US	4977802	
		WO 9101838				
JP	3086834	NONE				
AU	8942432	DE 68911473	EP 366277	GB	8822857	
		JP 2218609				
US	5283236	NONE				
AU	8820051	EP 301392	JP 1050823			
						END OF ANNEX